

Findings from a household randomized controlled trial of hand washing and face masks to reduce influenza transmission in Bangkok, Thailand

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Background Evidence is needed on the effectiveness of non-pharmaceutical interventions (NPIs) to reduce influenza transmission.

Methodology We studied NPIs in households with a febrile, influenza-positive child. Households were randomized to control, hand washing (HW), or hand washing plus paper surgical face masks (HW + FM) arms. Study nurses conducted home visits within 24 hours of enrollment and on days 3, 7, and 21. Respiratory swabs and serum were collected from all household members and tested for influenza by RT-PCR or serology.

Principal Findings Between April 2008 and August 2009, 991 (16·5%) of 5995 pediatric influenza-like illness patients tested influenza positive. Four hundred and forty-two index children with 1147 household members were enrolled, and 221 (50·0%) were aged <6 years. Three hundred and ninety-seven (89·8%) households reported that the index patient slept in the parents' bedroom. The secondary attack rate was 21·5%, and 56/345

(16·3%; 95% CI 12·4–20·2%) secondary cases were asymptomatic. Hand-washing subjects reported 4·7 washing episodes/day, compared to 4·9 times/day in the HW + FM arm and 3·9 times/day in controls ($P = 0\cdot001$). The odds ratios (ORs) for secondary influenza infection were not significantly different in the HW arm (OR = 1·20; 95% CI 0·76–1·88; $P = 0\cdot442$), or the HW + FM arm (OR = 1·16; 95% CI 0·74–1·82; $P = 0\cdot525$).

Conclusions Influenza transmission was not reduced by interventions to promote hand washing and face mask use. This may be attributable to transmission that occurred before the intervention, poor facemask compliance, little difference in hand-washing frequency between study groups, and shared sleeping arrangements. A prospective study design and a careful analysis of sociocultural factors could improve future NPI studies.

Keywords face mask, hand washing, influenza, non-pharmaceutical intervention, Thailand.

Background

Since 1997, outbreaks of avian influenza A (H5N1) among domestic poultry and sporadic human infections have generated global concern for an impending influenza pandemic. In response, the World Health Organization (WHO) and countries around the world began to consider options to respond to the pandemic threat including vaccines, antiviral medications, and non-pharmaceutical interventions (NPI). Considerable obstacles exist for the timely development of effective and affordable strain-specific pan-

demic vaccines.^{1,2} High levels of adamantane resistance among influenza A (H3N2) and A (H5N1) viruses^{3,4} widespread resistance to neuraminidase inhibitors among seasonal influenza A (H1N1) viruses^{5,6}, and reports of neuraminidase resistant influenza A (H5N1) variants also raised questions about the role of influenza antiviral drugs during a pandemic.^{7–9} At the same time, the evidence base supporting the effectiveness of personal protective measures such as hand washing and face mask use is insufficient.^{10–12} In early 2009, a novel reassortant influenza A (H1N1) virus unexpectedly emerged in the Americas and rapidly spread

globally to prompt the WHO to declare a pandemic on June 11th.¹³ The 2009 influenza A (H1N1) pandemic further underscored the need to improve the evidence base for NPI recommendations to control the spread of novel influenza A viruses.^{14–17} We conducted the household influenza transmission study (HITS) to estimate the efficacy of interventions to promote the use of hand washing alone, and hand washing with face mask use to decrease influenza virus transmission in households.

Study design

We prospectively identified pediatric patients who sought care for influenza-like illness (ILI) at the outpatient department of the Queen Sirikit National Institute of Child Health (QSNICH) in Bangkok, the largest public pediatric hospital in Thailand. For children <2 years of age, ILI was defined as fever >38°C and one or more of the following symptoms; nasal discharge/congestion, cough, conjunctivitis, respiratory distress (tachypnea, retractions), sore throat, and new seizure. For children aged ≥2 years, ILI was defined as fever >38°C and cough or sore throat in the absence of another explanation.¹⁸ Eligible patients hereafter referred to as index cases were children aged 1 month through 15 years, residents of the Bangkok metropolitan area, and had an onset of illness <48 hours before respiratory specimens tested positive for influenza by a rapid influenza diagnostic test (RIDT) that was later confirmed by qualitative real-time RT-PCR (rRT-PCR). Children at high risk for severe influenza complications (e.g., chronic lung disease, renal disease, and long-term aspirin therapy) and those treated with influenza antiviral medications were excluded. Eligible index cases' households must have had at least two other members aged ≥1 month who planned to sleep inside the house for a period of at least 21 days from the time of enrollment. Households with any member reporting an ILI that preceded the index case by 7 days or less and households where any member had received influenza vaccination during the preceding 12 months were excluded. All subjects aged 18 years and older provided written consent to participate, and proxy written consent from parents or legal guardians was obtained for children. Households were compensated for their time to participate in the study with approximately US \$60 in Thai baht. The study was approved by the QSNICH and the US CDC institutional review boards and was funded by the US CDC. Laboratory testing costs were partially supported by the Global Emerging Infections Surveillance and Response System, a Division of the US Armed Forces Health Surveillance Center.

Enrolled families were randomized to one of the three study arms in a 1:1:1 ratio. Randomization was achieved using a block randomization method using a list of blocks each with 12 household IDs, four of which were assigned

to each of the three study arms. A study coordinator assigned each household to one study arm after consent was obtained. Recruiting clinicians were blinded to the allocation of the specific intervention. The control group received nutritional, physical activity, and smoking cessation education. Intervention group 1 households received hand-washing education and a hand-washing kit that included a graduated dispenser with standard unscented liquid hand soap (Teepol brand. Active ingredients: linear alkyl benzene sulfonate, potassium salt, and sodium lauryl ether sulfate). Intervention group 2 households received hand-washing education and the hand-washing kit, and a box of 50 standard paper surgical face masks and 20 pediatric face masks (Med-con company, Thailand #14IN-20AMB-30IN). Specifics of the intervention education have been published previously by Kaewchana *et al.*¹⁹ Briefly, at the initial home visit to intervention 1 and 2 households, we provided intensive, interactive hand-washing education and individual hand-washing training that conveyed messages about 'why to wash', 'when to wash', and also 'how to wash' in seven hand-washing steps described in Thailand Ministry of Public Health (MOPH) guidelines. In intervention group two households, we provided education of the benefits of face mask wearing and instruction on the appropriate technique of wearing face masks to household members. We did not suggest that members wear the face masks while eating or sleeping as this was not deemed practical and that it could hinder breathing in an ill child. When prompted with specific questions by family members during subsequent home visits, study nurses provided impromptu education and training to reinforce the messages delivered during the first visit.

Following randomization of an enrolled household, a study nurse collected baseline data and scheduled a home visit to be completed within 24 hours (Day 0/1). The study nurse visited the family again on days 3, 7, and 21 following enrollment. Family members were asked to maintain daily records of symptoms, hand-washing frequency of >20 seconds duration, and duration of face mask use. Time in minutes spent within 1 m of the index case during their illness was also recorded. In addition, information on the amount of household liquid soap and number of face masks used was collected at study visits. Soap was replaced as needed. Subjects in the control arm were asked about their hand washing and face mask use during the Day 7 home visit to capture the information without influencing these behaviors during the study period. Nasal and throat swab specimens were obtained on Days 0/1, 3, and 7 from the index case and all household members. Specimens were aliquoted and tested by qualitative rRT-PCR to detect influenza viral RNA. Blood specimens were collected from each consenting household member on Day 0/1 and again on Day 21 for serological testing by hemagglutinin

inhibition (HI) assay to identify asymptomatic infection and correlate with qualitative PCR results. A fourfold rise in HI antibody titer in paired sera was considered to be evidence of an acute influenza virus infection.

Statistical methods

With a significance level of 0·05, anticipating a secondary attack rate (SAR) of 15% and a within-household correlation of 0·2, we specified a sample size of 1200 household contacts in 400 households in each arm to permit 80% power to detect a 30% reduction in the SAR (intervention effect). To evaluate and compare SARs, we estimated 95% confidence intervals (CIs) using a cluster bootstrap technique with 1000 resamples and chi-square tests adjusting for potential within-household correlation.^{20,21} The primary study outcome was laboratory-confirmed secondary influenza virus infections among household members described as the SAR. A secondary influenza virus infection was defined as a positive rRT-PCR result on Days 3 or 7 or a fourfold rise in influenza HI antibody titers with the virus type and subtype matching the index case. We also evaluated the SAR for influenza-like illness (ILI) defined by the WHO as fever plus cough or sore throat, based on self-reported symptoms. Household members that tested positive for influenza on Day 1 were considered to be 'co-index' infections. The analysis of primary outcomes was by intention to treat in the cohort of households without co-index cases. We also analyzed SARs in a subset of households where the intervention was implemented within 48 hours of the onset of symptoms in the index case. Student's *t*-test was used to compare approximately normally distributed continuous variables. The chi-square test was used to evaluate association between categorical variables on the outcome of secondary infections. The chi-square tests comparing the individual household member risk of infection (the individual level SAR) were adjusted for correlation of outcomes within households.²² We fitted logistic regression models using the generalized estimating equations (GEE) approach to adjust for within-household correlation. We assumed that other household members within a household have the same risk of acquiring influenza virus infection from the index case, and for this reason, we used the exchangeable correlation structure in the GEE model. To account for correlation of outcomes within households, the logistical model used with the GEE produces odds ratios (ORs), which in this setting over estimates the relative risk. We included household-level and individual-level characteristics in multi-variable logistic regression analyses to adjust for variables relevant for secondary influenza virus infection.

Laboratory methods

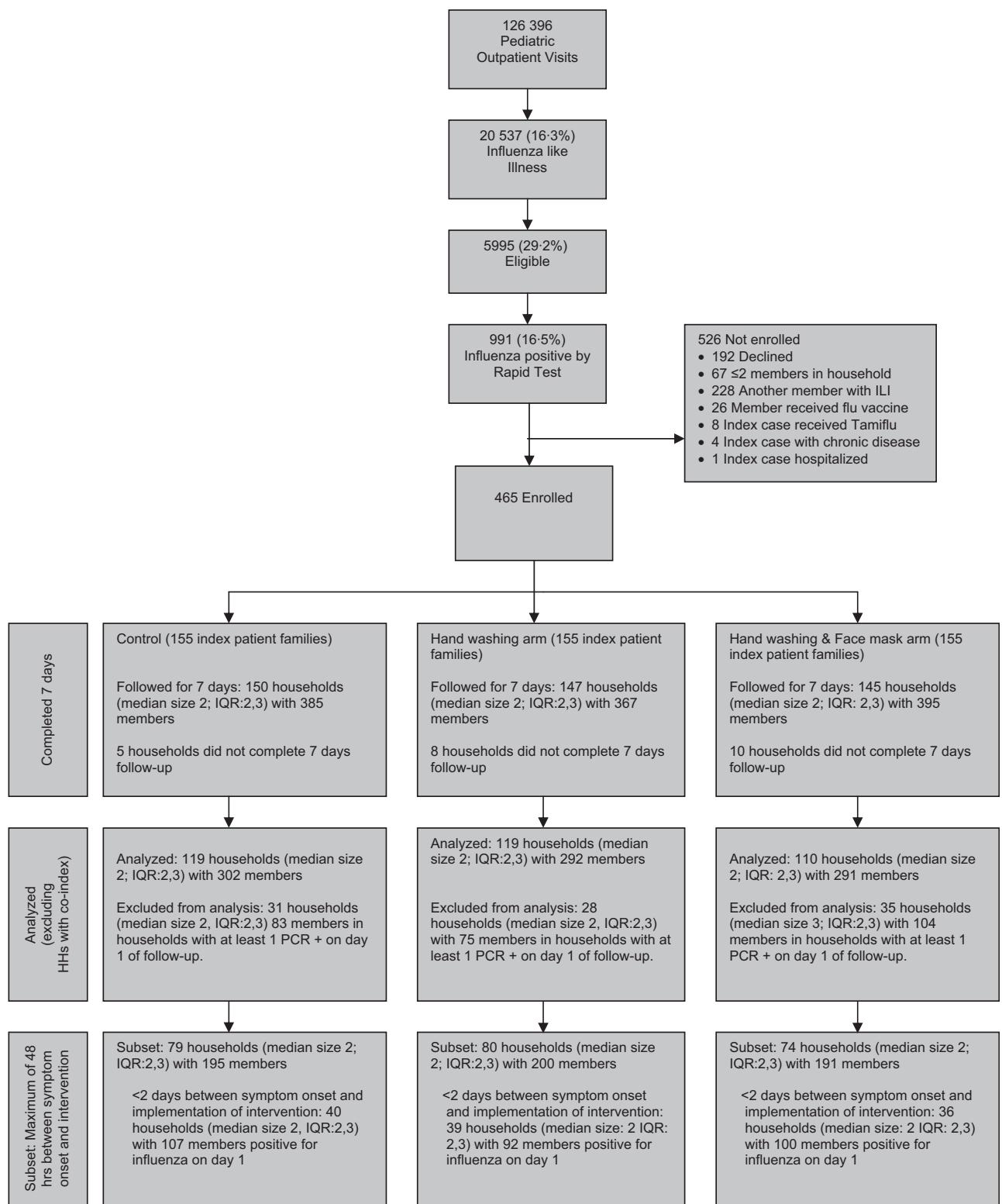
To identify index patients in the pediatric outpatient department, a foam-tipped nasal swab provided by the

manufacturer was tested for influenza using the QuickVue Influenza A + B rapid diagnostic kit (Quidel Co., San Diego, CA, USA). If the RIDT was positive, one additional nasal swab and one throat swab were collected from the ill child and inserted into a 15-ml container of viral transport media (VTM) Remel M4RT Multi-Microbe Media (RE-MEL, Lenexa, KS, USA), snapped off at the perforation and placed on wet ice in a portable cooler or directly in a standard 4°C refrigerator. To confirm the RIDT results, swab specimens from the index cases were sent the same day on wet ice to the Armed Forces Institute of Medical Sciences (AFRIMS) in Bangkok, aliquoted and tested by rRT-PCR for influenza viral RNA. The remaining samples were stored at -70°C.

During each subsequent home visit, one nasal swab and one throat swab were collected from the index case and from all household contacts. Both swab specimens were immediately placed in a single vial of VTM and then on wet ice or cold packs and delivered to the AFRIMS laboratory the same day or stored at 4°C at QSNICH overnight until delivery to the laboratory the following morning. The specimens were then aliquoted and stored at -70°C until processed for rRT-PCR. Blood samples were collected on Days 1 and 21 in a serum separator tube. The tubes were delivered to the laboratory at room temperature, and serum was separated by low-speed centrifugation (10 min 100 × g), aliquoted, and frozen at -70°C. Viral RNA was extracted from 140 µl of inoculated VTM using the QIAamp Viral RNA Mini kit method (QIAGEN, Valencia, CA, USA) according to the manufacturers' recommendations. All respiratory samples from index cases and household members were first tested with universal influenza A and universal influenza B primers and probes. Samples positive for universal influenza A were then tested with H1- (seasonal and also for 2009 H1N1) and H3-specific probes and primer sequences developed by and under material transfer agreement with the US Centers for Disease Control using the Rotogene 3000 Real-time PCR thermocycling instrument (Cybeles, Australia). In June 2009, primers for 2009 influenza A (H1N1) were obtained from US CDC and introduced into the testing algorithm. Approximately 5 ml of serum collected at Days 1 and 21 were tested for antibody seroconversion using the WHO Haemagglutinin Inhibition kit (provided by US CDC Atlanta) per manufacturers' recommendations using 0·75% guinea pig red blood cells resuspended in PBS and BSA. Seroconversion was defined as a fourfold rise in HI titer between paired sera for any of the antigens assayed.

Results

Of 5995 eligible pediatric ILI outpatients between April 9, 2008 and August 13, 2009, 991 (16·5%) tested positive for

**Figure 1.** Enrollment process.

influenza by RIDT (Figure 1). Four hundred and sixty-five influenza-positive children were enrolled, and 442 (95%) households with 1147 members completed three home visits (Days 0/1, 3, and 7). Two hundred and twenty-one (50·0%) index patients were aged <6 years, and 384 (86·9%) were aged <11 years (median age 5·5 years). The median age of household contacts was 34 years (ICR 24–42). Two hundred and fifty households (56·6%) were enrolled prior to June 1, 2009, and 192 households (43·4%) were enrolled after that date, the putative start of the 2009 H1N1 pandemic in Thailand. The index cases of 122 (64%) households enrolled after June 1st tested positive for 2009 pandemic influenza A (H1N1) virus with the remainder mostly influenza A (H3N2) virus infections (Figure 2). Three hundred and forty-four (77·8%) households received the first visit on the same day they were enrolled, and the remaining households were visited the day after enrollment. One thousand one hundred and twenty-six (98%) household members provided three sets of respiratory specimens, and 938 (82%) members provided paired sera. Household size ranged between 3 (by design in the inclusion criteria) and 14. Three hundred and six (69%) households had ≤4 members. In 263 (59·5%) households, the index case was the only child. Three hundred and ninety-seven (89·8%) households reported that the index patient slept in the same bedroom as the parents. The majority of families enrolled in HITS resided in small, one-bedroom, low-rent apartments without air conditioning systems in urban Bangkok.

Across all study arms, 343 (29·9%) of 1147 family members in the 442 households had a laboratory-confirmed influenza virus infection with the same influenza type and subtype as the index case (309 by rRT-PCR and 34 addi-

tional cases identified by serology). Six household members had influenza infections that differed by type or subtype from the index case. Only 165 (48%) met the WHO criteria for ILI. Fifty-six of the 343 (16·3%; 95% CI 12·4–20·2%) influenza-positive individuals reported no symptoms of illness. Compared to the symptomatic cases, asymptomatic cases tended to be older, with mean age of 38 years compared to 30 years for the symptomatic cases (t -test P -value = 0·0004). Asymptomatic cases were similar to symptomatic cases in the distribution of influenza type/subtype as the symptomatic cases ($\chi^2 P = 0·69$). One hundred and thirteen infections in 94 households were co-index cases identified on the Day 1 home visit. We excluded these 94 households from the analysis, because the true index case could not be established and these infections had occurred before interventions had been implemented. Therefore, the intent to treat analysis included 348 households and 885 members. Households and individuals in the control and intervention arms did not differ significantly with respect to important covariates at the household or individual level (Table 1a,b).

Intention to treat analysis

The overall SAR across study arms among all household members was 22% (190 of 885; 95% CI 19–24%) (Table 2). The individual-level SAR for laboratory-confirmed influenza in the control, hand-washing arm, and hand washing plus face mask arm, 19%, 23%, and 23%, respectively, was not statistically different (adjusted $\chi^2 P = 0·63$). In the subset of households where the intervention was applied within 48 hours of index case illness onset, the SAR for laboratory-confirmed influenza in the control arm (23%) was more similar to the hand-washing arm (24%) although still the lower than facemask plus hand-washing arm (27%). The difference between the arms remained not significant (adjusted $\chi^2 P = 0·79$). The SAR for laboratory-confirmed influenza was greatest in households where the index case was 4–5 years of age (31%; 95% CI 23–40%) and lowest in households with an index case aged 11–14 years (18%; 95% CI 12–26%) (data not shown). Among 348 households, 144 (41·4%) had at least one secondary influenza virus infection. The SAR for laboratory-confirmed influenza at the household level (# positive/# contacts per arm) in the control (39%), hand washing (44%), and hand washing plus face mask arms (42%) was not statistically different ($\chi^2 P = 0·73$ data not shown). One hundred and twenty-seven household contacts (14%; 95% CI 12–17%) had ILI. The SAR for ILI was 9% in the control arm, 17% in the hand-washing arm, and 18% in the face mask plus hand-washing arm. The SAR for ILI at the household level was 22% in the control arm, 35% in the hand-washing arm, and 35% in the hand washing plus face mask arm ($\chi^2 P = 0·03$ data not shown).

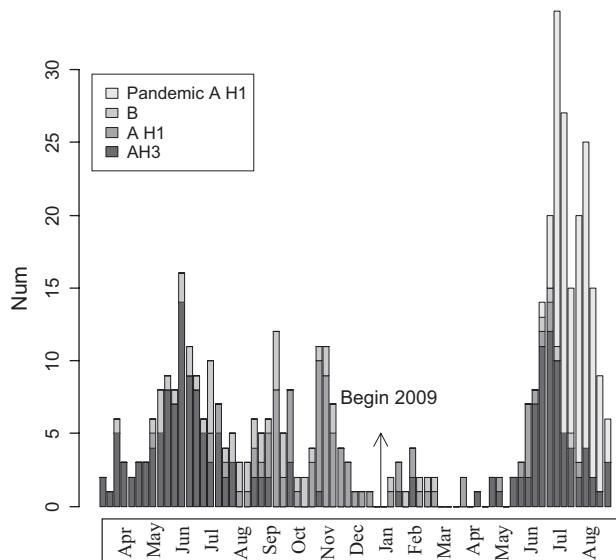


Figure 2. Distribution of influenza subtypes in index cases by study month.

Table 1. Distribution of covariates by primary exposure at (a) household-level Household Influenza Transmission Study (HITS) study arm and (b) individual-level HITS study arm

Household level	All (N = 348)	Control (N = 119)	Hand wash (N = 119)	Face mask (N = 110)
(a)				
Index case influenza subtype				
A(H1) seasonal	68 (19·5)	22 (18·5)	24 (20·2)	22 (20·0)
A (H3)	129 (37·1)	45 (37·8)	42 (35·3)	42 (38·2)
Type B	41 (11·8)	13 (10·9)	14 (11·8)	14 (12·7)
Pandemic A(H1N1) 2009	110 (31·6)	39 (32·8)	39 (32·8)	32 (29·1)
P for chi-square in referent to control			0·973	0·924
Index gender				
Female	156 (44·8)	50 (42·0)	59 (49·6)	47 (42·7)
Male	192 (55·2)	69 (58·0)	60 (50·4)	63 (57·3)
P for chi-square in referent to control			0·242	0·913
Index age category				
0–1	49 (14·1)	25 (21·0)	12 (10·1)	12 (10·9)
2–3	66 (19·0)	17 (14·3)	26 (21·9)	23 (20·9)
4–5	53 (15·2)	15 (12·6)	19 (16·0)	19 (17·3)
6–10	131 (37·6)	47 (39·5)	46 (38·7)	38 (34·6)
11–15	49 (14·1)	15 (12·6)	16 (13·5)	18 (16·4)
P for chi-square in ref to control			0·138	0·146
Household size (includes index case)				
3	120 (34·5)	42 (35·3)	46 (38·7)	32 (29·1)
4	123 (35·3)	44 (37·0)	38 (31·9)	41 (37·3)
5	50 (14·4)	18 (15·1)	19 (16·0)	13 (11·8)
6 +	55 (15·8)	15 (12·6)	16 (13·5)	24 (21·8)
P for chi-square in referent to control			0·878	0·262
Households with other children (<16 years)				
No other children	216 (62·1)	78 (65·6)	75 (63·0)	63 (57·3)
1 other child	118 (33·9)	37 (31·1)	41 (34·5)	40 (36·4)
2–3 other children	14 (4·0)	4 (3·4)	3 (2·5)	7 (6·4)
P for chi-square in referent to control			0·821†	0·336
Index patient sleeping arrangement				
Own room	15 (4·3)	5 (4·2)	5 (4·2)	5 (4·6)
Shares with other children	13 (3·7)	5 (4·2)	3 (2·5)	5 (4·6)
Shares with parents	315 (90·5)	107 (89·9)	109 (91·6)	99 (90·0)
Other	5 (1·4)	2 (1·7)	2 (1·7)	1 (0·91)
P for chi-square in referent to control			0·930†	1·00
(b)				
Individual level	All (N = 885)	Control (N = 302)	Hand wash (N = 292)	Face mask (N = 291)
Relationship to index case				
Parent	535 (60·5)	178 (58·9)	183 (62·7)	174 (59·8)
Sibling	137 (15·5)	41 (13·6)	46 (15·8)	50 (17·2)
Grandparent	113 (12·8)	49 (16·2)	33 (11·3)	31 (10·7)
Cousin	28 (3·2)	11 (3·6)	7 (2·4)	10 (3·4)
Other	72 (8·1)	23 (7·6)	23 (7·9)	26 (8·9)
P for chi-square in referent to control			0·379	0·286
Gender				
Female	523 (59·1)	176 (58·3)	175 (59·9)	172 (59·1)
Male	362 (40·9)	126 (41·7)	117 (40·1)	119 (40·9)
P for chi-square in referent to control			0·682	0·838

Table 1. (Continued)

Individual level	All (N = 885)	Control (N = 302)	Hand wash (N = 292)	Face mask (N = 291)
Age of household contacts				
0–15	149 (16·8)	46 (15·2)	47 (16·1)	56 (19·2)
16–30	188 (21·2)	70 (23·2)	61 (20·9)	57 (19·6)
31–50	445 (50·3)	151 (50·0)	147 (50·3)	147 (50·5)
51+	103 (11·6)	35 (11·6)	37 (12·7)	31 (10·7)
P for chi-square in referent to control			0·903	0·493
Time spent within 1 m of child (quartile)				
Q1 (least)	223 (25·2)	77 (25·5)	72 (24·7)	74 (25·4)
Q2	222 (25·1)	72 (23·8)	73 (25·0)	77 (26·5)
Q3	219 (24·8)	76 (25·2)	66 (22·6)	77 (26·5)
Q4 (most)	221 (25·0)	77 (25·5)	81 (27·7)	63 (21·7)
P for chi-square in referent to control			0·847	0·698

†P-value from Fisher's exact Test.

Multivariable analysis

The adjusted OR for a secondary influenza virus infection among household members in the hand-washing arm was not statistically different from the control arm (1·20; 95% CI 0·76–1·88; P = 0·442). Neither was the adjusted OR for the hand washing plus face mask arm (1·16; 95% CI 0·74–1·82; P = 0·525) (Table 3). As a *post hoc* hypothesis, we asked whether the pandemic strain of influenza was more pathogenic than seasonal influenza. Households with index cases infected with seasonal influenza virus strains were slightly less likely to experience a secondary infection compared to those with an ill pandemic influenza index case, although this was not statistically significant. Time spent in close proximity (<1 m) from the index case was a strong predictor for a secondary influenza virus infection with an OR of 2·0 in the group reporting the highest exposure (95% CI 1·19–3·37; P = 0·009). We hypothesized that rapid implementation of the interventions would increase the protective efficacy of hand washing and face masks. Therefore, we analyzed data from a subset of households where the intervention was implemented within 48 hours of the onset of symptoms in the index case. In this subset of 233 households with 586 members, the OR for a secondary influenza virus infection among household members in the hand-washing arm was 1·06 (95% CI 0·62–1·82; P = 0·82). Similarly, the OR for the hand washing plus surgical face mask use arm was 1·15 (95% CI 0·68–1·93; P = 0·61) (Table 4).

Relative to the control group, the ORs for ILI among household members in the hand-washing arm (2·09; 95% CI 1·25, 3·50; P = 0·005) and hand washing plus face mask arm (2·15; 95% CI: 1·27, 3·62; P = 0·004) were twofold in the opposite direction from the hypothesized protective effect (Table 3). These results were similar among the subset of households where the intervention occurred within

48 hours of the onset of symptoms in the index case (Table 4).

Adherence

Subjects in the control arm reported an average of 3·9 hand-washing episodes/day (on Day 7) while subjects in the hand washing arm reported an average of 4·7 hand-washing episodes/day (95% CI 4·3–5·0; P = 0·002 compared to controls), and subjects in the hand washing plus face mask arm reported 4·9 episodes/day (95% CI 4·5–5·3; P < 0·00011 compared to controls). In the intervention arms, parents had the highest reported daily hand-washing frequency (5·7 95% CI: 5·3, 6·0) followed by others (4·8 95% CI 4·3, 5·3), siblings (4·3 95% CI: 3·7, 4·8) and the index cases (4·1 95% CI: 3·8, 4·4) (Figure 3). There was no difference in the average amount of soap used in a week in the hand-washing arm (54 ml per person) and the hand washing plus face mask arm (58·1 ml per person) (P = 0·15). Two hundred and eighty-nine subjects in the face mask arm used an average of 12 masks per person per week (median 11, IQR: 7, 16) and reported wearing a face mask a mean of 211 minutes/day (IQR = 17–317 minutes/day). Parents wore their masks for a median of 153 (IQR = 40–411) minutes per day, far more than other relations (median 59; IQR = 9–266), the index patients themselves (median 35; IQR: 4–197), or their siblings (median 17; IQR: 6–107) (Figure 4). We note that differences in average usage may be an attenuated measure of appropriate use in relation to the actual unmeasured exposure risk such as proximity to the index case.

The first wave of the 2009 influenza A (H1N1) pandemic in June 2009 complicated the study. In response to the pandemic, the Thailand MOPH implemented extensive national hand and respiratory hygiene educational

Table 2. Secondary attack rate of RT-PCR or serologically confirmed or influenza-like illness (ILI) among household contacts

Time between symptom onset and intervention	Study arm		All analytic						Household members = 885	
	Control Household members = 302		Hand-washing Household members = 292			Facemask and hand washing Household members = 291			Household members = 885	
	Cases	n SAR (%)	Cases	n SAR (%)	Cases	n SAR (%)	Adjusted Chi-sq *	Cases	n SAR (%)	95% CI †(%)
At any time										
By PCR or serology	58	0·19 (0·14, 0·24)	66	0·23 (0·18, 0·28)	66	0·23 (0·17, 0·28)	0·63	190	0·21 (0·19, 0·25)	
ILI	26	0·09 (0·06, 0·12)	50	0·17 (0·13, 0·22)	51	0·18 (0·13, 0·22)	0·01	127	0·14 (0·12, 0·17)	
Intervention within 48 hours										
By PCR or serology	195		200		191		Members = 586			
ILI	45	0·23 (0·17, 0·30)	48	0·24 (0·18, 0·31)	51	0·27 (0·19, 0·34)	0·79	144	0·25 (0·21, 0·29)	
	18	0·09 (0·06, 0·13)	40	0·20 (0·14, 0·26)	36	0·19 (0·13, 0·25)	0·02	94	0·16 (0·13, 0·20)	

*Pearson chi-square for difference among the three intervention arms, adjusted for within-household correlation of 0·18 for the PCR or serology outcome and 0·05 for the ILI outcome.
†The CIs were calculated using the cluster bootstrap method.

campaigns that increased these behaviors in the control arm households. In an analysis of hand-washing behavior of 207 control group subjects enrolled before June 1, 2009 and 162 enrolled after that date (the approximate onset of the first wave of the 2009 pandemic), mean reported hand-washing episodes per day increased from 3·7 to 4·1 ($P = 0·09$). Mean reported daily face mask use also increased during June to August 2009. When asked during the Day 7 home visit, 65 of 370 (17·6%) control family members reported using used facemasks during the study week and 44 (67·7%) of these were members of families enrolled after June 1, 2009. Among index cases in the control arm, 3 of 83 (4%) enrolled before the pandemic reported using a mask during the study week, compared to 29 of 56 (52%) of index patients enrolled after June 1, 2009 ($P \leq 0·001$).

Discussion

We report the largest study to date of the efficacy of interventions to promote hand washing and hand washing plus face mask use to reduce influenza transmission. Influenza transmission among household members of a confirmed index case was not reduced by promotion of hand washing and face mask use. In contrast, a similar study in Hong Kong reported that when hand washing and face mask intervention were introduced within 36 hours of the onset of symptoms on the index patient, these interventions seemed to reduce influenza transmission although no difference in secondary transmission was observed in the intent to treat analysis in that study.²³ There are several potential explanations for the lack of significant effects observed in our study. Ninety percent of ill index case children in our study slept in the same bedroom as their parents, an arrangement that is uncommon in Hong Kong (Ben Cowling, personal communication). Given that masks were not worn while sleeping, this prolonged and close exposure during periods of high viral shedding may have overcome any potential protective effects from the interventions. In addition, transmission from the index child to the parent may have occurred very early in the child's illness before interventions could be initiated.

The first wave of the 2009 influenza A (H1N1) pandemic in June 2009 introduced new challenges to our study. In response to the pandemic, the Thailand MOPH implemented a national hand and respiratory hygiene educational campaign that increased hand washing and face mask use in control arm households. Overall, subjects in the control arm reported washing their hands only slightly less often (3·9 episodes/day) than participants in the intervention groups (4·7 in hand washing; 4·9 in hand wash plus face mask). While these differences were statistically

Table 3. Individual-level analysis in the analytic subset of 885 members in 348 households (94 co-index households removed)

	Influenza by PCR and serology			ILI		
	OR	95%CI	P-value	OR	95%CI	P-value
Control	1·00			1·00		
Hand washing	1·20	(0·76, 1·88)	0·442	2·09	(1·25, 3·50)	0·005
Hand wash + Face mask	1·16	(0·74, 1·82)	0·525	2·15	(1·27, 3·62)	0·004
Index case subtype						
2009 pandemic H1N1	1·00			1·00		
Seasonal influenza A and B	0·92	(0·74, 1·88)	0·695	0·87	(0·55, 1·38)	0·553
Index gender						
Female	1·00			1·00		
Male	1·27	(0·87, 1·85)	0·211	1·09	(0·73, 1·63)	0·681
Index age						
<2	1·00			1·00		
2–3	1·17	(0·63, 2·18)	0·619	1·46	(0·68, 3·12)	0·333
4–5	1·75	(0·90, 3·38)	0·097	1·76	(0·83, 3·76)	0·143
6–10	0·96	(0·51, 1·80)	0·889	0·88	(0·42, 1·85)	0·745
11–15	0·96	(0·46, 2·00)	0·909	0·80	(0·35, 1·85)	0·598
Gender						
Female	1·00			1·00		
Male	1·02	(0·73, 1·41)	0·927	0·88	(0·59, 1·31)	0·533
Proximity to index case						
Q1	1·00			1·00		
Q2	1·19	(0·74, 1·93)	0·474	0·93	(0·54, 1·59)	0·779
Q3	1·95	(1·16, 3·29)	0·012	0·94	(0·52, 1·70)	0·827
Q4	2·00	(1·19, 3·37)	0·009	1·20	(0·65, 2·22)	0·600
Age of member						
0–15	1·00			1·00		
16–30	0·61	(0·36, 1·03)	0·067	0·83	(0·45, 1·51)	0·537
31–50	0·74	(0·48, 1·15)	0·181	0·83	(0·49, 1·41)	0·487
≥51	0·64	(0·35, 1·17)	0·149	0·46	(0·20, 1·04)	0·063

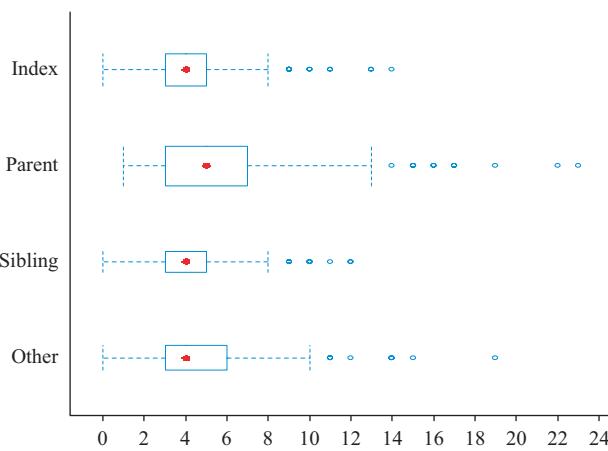
significant, they were unlikely to be clinically significant in terms of reducing transmission. Finally, the Hong Kong study provided both alcohol hand rub and liquid soap while our study used only liquid soap. It is conceivable that the addition of alcohol hand rub may have increased the efficacy of the hand-washing intervention in that study.²⁴ Alcohol hand rub was not employed in this study because these products are not widely available or affordable to most of the world's population.

The Hong Kong study found protective effects in households where interventions were implemented within 36 hours of symptom onset in the index patient. While we did not detect reductions in overall household SAR, the OR trended in the direction of a protective effect of NPI in the secondary analysis of SAR of influenza virus infection confirmed by rRT-PCR or serology among households that received the interventions within 48 hours of the onset of illness in the index case. As expected, the risk of infection increased with time spent in proximity to the index case. These findings have potential implications for targeted infection control recommendations. The SAR among

control arm household members was 19%, while the SAR in the Hong Kong study was 10%.²³ Young children shed higher quantities of influenza virus²⁵, and in our study, 48·3% of the index cases were children under 6 years of age compared with 17% of index cases in the Hong Kong study, a factor which may explain the much higher SAR we observed. Interestingly, pandemic influenza virus infection in the index case was not associated with an increased risk of secondary influenza transmission compared to seasonal influenza infections. The estimates from the multivariate model for clinically defined ILI indicate an elevated risk in the intervention arms but the monotonic increase in risk observed with increasing proximity in the laboratory-confirmed multivariate model is not present. The ORs for clinically defined ILI are therefore questionable and probably the result of sensitization bias such that subjects in the intervention arms may have been more likely to report perceived symptoms in a way that did not occur in the control arm. Incidentally, the ORs for the clinically defined outcome in the intervention arms of the Hong Kong study also suggest an increased risk. This underscores the value

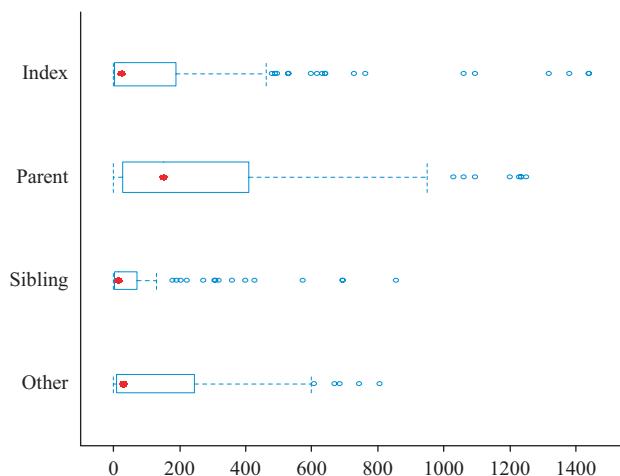
Table 4. Individual-level analysis in households where intervention occurred within 48 hours of index case symptom onset

	233 households (586 members) influenza by PCR or serology			233 households (586 members) ILI		
	OR	95%CI	P-value	OR	95%CI	P-value
Control	1·00			1·00		
Hand Washing	1·06	(0·62, 1·82)	0·819	2·38	(1·32, 4·29)	0·004
Hand wash + Face Mask	1·15	(0·68, 1·93)	0·609	2·16	(1·14, 4·07)	0·018
Index case subtype						
2009 pandemic H1N1	1·00			1·00		
Seasonal influenza A and B	0·99	(0·60, 1·64)	0·978	0·98	(0·57, 1·70)	0·957
Index Gender						
Female	1·00			1·00		
Male	1·13	(0·73, 1·75)	0·592	0·99	(0·61, 1·61)	0·972
Index age category						
<2	1·00			1·00		
2–3	0·89	(0·40, 1·95)	0·766	1·21	(0·40, 3·69)	0·738
4–5	1·54	(0·70, 3·38)	0·279	2·33	(0·82, 6·58)	0·111
6–10	0·92	(0·42, 2·01)	0·844	1·22	(0·43, 3·44)	0·709
11–15	0·92	(0·38, 2·21)	0·854	1·06	(0·35, 3·22)	0·921
Gender						
Female	1·00			1·00		
Male	1·02	(0·70, 1·49)	0·901	0·88	(0·60, 1·58)	0·923
Proximity to index case						
Q1	1·00			1·00		
Q2	1·21	(0·70, 2·07)	0·494	1·24	(0·65, 2·36)	0·523
Q3	1·70	(0·91, 3·19)	0·096	1·10	(0·54, 2·26)	0·794
Q4	1·99	(1·06, 3·72)	0·031	1·50	(0·71, 3·18)	0·284
Age of member						
0–15	1·00			1·00		
16–30	0·60	(0·33, 1·10)	0·101	0·96	(0·48, 1·94)	0·920
31–50	0·67	(0·42, 1·08)	0·107	0·63	(0·35, 1·13)	0·121
≥51 plus	0·72	(0·36, 1·44)	0·348	0·38	(0·14, 1·02)	0·055

**Figure 3.** Mean reported hand-washing episodes per day.

of objective laboratory measures in the study of interventions to prevent influenza.

Our study has limitations and faced a number of challenges inherent in the introduction and measurement of

**Figure 4.** Mean reported minutes wearing mask per day.

behavioral interventions inside the home. The study was not designed to determine exposure risk epidemiologically and influenza virus transmission risk outside the household

setting from exposure to ill non-household members. The operation of the study was complicated by the arrival of the 2009 H1N1 influenza pandemic in June 2009 and the subsequent national hygiene campaign that prompted behavioral changes in the control group. While delays in the implementation of the interventions are an inherent flaw in this study design, alternative designs require much larger sample sizes and increased costs. Our study was not designed to assess other potentially important parameters such as air flow, air quality, and other environmental factors that may play a role in household influenza transmission. Poor adherence to the interventions, especially among index cases and their younger siblings, may have further contributed to an underestimation of the true effects of hand washing or face mask use. In a recent study by McIntyre and colleagues, per-protocol (actual use) analysis suggested a protective effect against ILI in adherent face-mask users, but, again, reported no benefit in the intent to treat analyses.²⁶

Our findings should not be interpreted to conclude that hand washing or face mask use are not potentially useful public health measure to prevent infections other than influenza, but they do provide a potent example of the importance of understanding the dynamic and complex relationship between public health recommendations, local social customs and individual behavior, and their application for preventing transmission of specific pathogens. Indeed, hand washing has been shown to be effective in reducing respiratory infection in school, community, and military settings.^{27–29} Careful analysis of sociocultural factors will improve future non-pharmaceutical intervention studies and facilitate more effective implementation of public health recommendations to reduce influenza transmission. In the meantime, increased efforts are needed to provide for implementation of influenza vaccine programs in low- and middle-income countries as the primary means to decrease the number of severe illnesses and deaths from influenza.

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